MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

*April–June 2020—Volume 135, pp. 309–313*

https://doi.org/10.5248/135.309

# *Golovinomyces asperifolii:* first record in China and *Bothriospermum chinense* as a new host

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Abstract—*Golovinomyces asperifolii* is described from the Qilian mountains in Qinghai province as a new record in China. The identification is supported by morphological characteristics and phylogenetic analysis of rDNA ITS. The fungus was collected on *Bothriospermum chinense*, which represents a new host for *G. asperifolii*.

Key words—*Ascomycota, Boraginaceae*, *Erysiphaceae*, powdery mildew. taxonomy

## Introduction

The Qinghai-Tibet Plateau in China, known as "the ridge of the world," has very special vegetation. *Bothriospermum chinense* is a Chinese endemic plant used as a traditional Chinese medicine for treating urinary tract infections. In autumn 2018, it was found to be heavily infected by a powdery mildew in the Qilian mountains, Qinghai. Based on the morphological characteristics of the asexual morph, the mildew was classified as a *Golovinomyces* sp. (Braun & Cook 2012). Molecular analyses were conducted to resolve the species identity. The internal transcribed spacer (ITS) regions were sequenced and compared with sequences in depositories. Based on sequence analysis of the rDNA ITS region, this species has been identified as *Golovinomyces asperifolii*.

This species has not previously been reported in China (Zheng & Yu 1987, Wu & Wu 1991, Chen 1993, Wang & al. 2002, Liu 2010, Bai 2015); our collection constitutes the first Chinese record of this species.

## Materials & methods

Living leaves of *Bothriospermum chinense* bearing the anamorph of a powdery mildew were collected in autumn 2018 in the Qilian Mountains in China. The herbarium specimens were kept in Herbarium of Plant Pathology at Qinghai University, Xining, Qinghai Province, China (QHU).

A sample was mounted in distilled water and examined using an Olympus CX31RTSF light microscope. A JEOL JSM-6360LV scanning electron microscope was used to observe the anamorph ultrastructure, particularly conidial surface features (according to Cook & al. 1997) and hyphal appressoria.

Genomic DNA was extracted from the mycelium and the asexual morph using Chelex-100 (Walsh & al. 1991, Hirata & Takamatsu 1996). The nuclear rDNA ITS region (including the 5.8S gene) was amplified via polymerase chain reaction (PCR); PM5 and PM6 (Takamatsu & Kano 2001) were used to amplify the ITS region.

PCR assays were conducted in a 50 µL final volume (Hirata & Takamatsu 1996) containing 27 µL of 2× BoisTaq PCR MasterMix, 1 µL of each primer, 1 µL of the extracted DNA, and 20 µL of $ddH_2O$ (Hirata & Takamatsu 1996). Thermal cycling in a BioRad PTC-200 thermal cycler comprised an initial denaturation step at 95°C for 5 min, 35 cycles at 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced the template DNA with $ddH_2O$. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

All DNA sequences were aligned using Clustal X 1.81 (Thompson & al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura & al. 2007). Branch robustness was assessed by bootstrap analysis with 1000 replicates.

## Taxonomy

***Golovinomyces asperifolii***(Erikss.) U. Braun & H.D. Shin,
Mycobiology 46(3): 198. 2018. Plate 1

Mycelium on leaves and stems, amphigenous, persistent, irregular white patches, thin, effuse; hyphae hyaline, smooth, 3-6 µm wide; hyphal appressoria nipple-shaped; conidiophores erect, 100-250 µm long; foot-cells

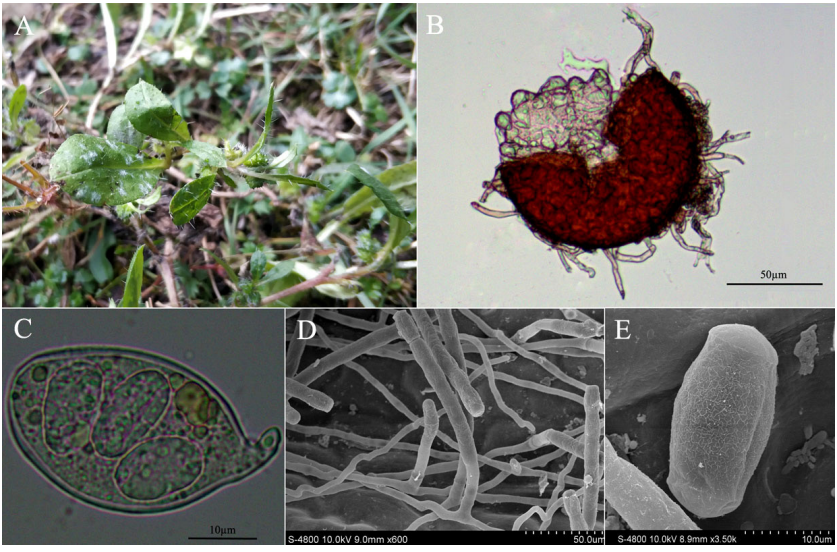


Plate 1. *Golovinomyces asperifolii* (QHU2018043): A. *Bothriospermum chinense* infected by a powdery mildew; B. Chasmothecia and appendages; C. Ascus and ascospores; D. Conidiophore, conidia, and hyphae; E. Conidia.

straight, cylindrical, 40–130 × 8–14 μm, followed by 1–3 shorter cells; conidia ovoid to doliiform, with irregular longitudinal or reticulate ridges under the SEM, 22–38 × 12–20 μm, length/width ratio 1.4–2.4, Chasmothecia gregarious or sometime scattered, subglobose to globose, 80–110 μm diam; peridium cells irregularly polygonal, 8–23 μm diam; appendages more or less equatorial and in the lower half of the chasmothecia, numerous, mycelioid, simple, unbranched, interlaced with each other and with the mycelium, 0.4–2 × times as long as the chasmothecial diam, 3–6 μm wide, septate, walls thin and smooth, hyaline or brown below and paler or colorless towards the tips; asci 6–12, with oil drops, ellipsoid-obovoid, clavate-saccate, 34–52 × 20–31 μm, short-stalked, 2–4-spored; ascospores ellipsoid-ovoid to almost globose, 10–19 × 10–18 μm, colorless.

Specimen examined: , 38°07′59″N 100°08′38″E, alt. 3280 m, on living leaves of *Bothriospermum chinense* Bunge (*Boraginaceae*), autumn 2018, L.C. Bai (QHU2018043; GenBank MK425651).

## Phylogeny

The ITS rDNA sequence comprising 474 total characters was deposited in GenBank. The new sequence was aligned with sequences from eleven

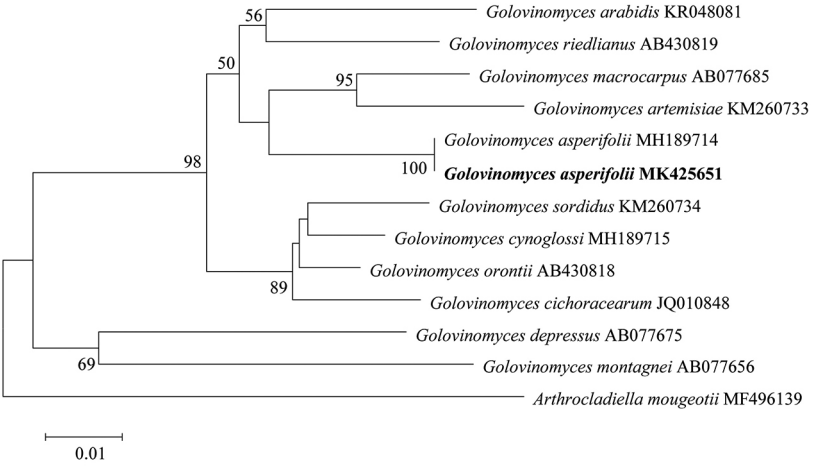


Plate 2. A neighbor-joining tree based on distances derived from sequences of ITS1, ITS2, and the 5.8S rRNA gene from 12 *Golovinomyces* sequences and an *Arthrocladiella mougeotii* outgroup sequence. The bar indicates a distance of 0.01.

*Golovinomyces* spp. and an outgroup sequence of *Arthrocladiella mougeotii* (Lév.) Vassilkov (Plate 2). The ITS phylogenetic tree placed the Chinese specimen and *Golovinomyces asperifolii* MH189714 (from Germany) in a strongly supported clade with 100% bootstrap support, indicating that these two sequences are conspecific.

## Discussion

*Golovinomyces asperifolii* was described worldwide on hosts of the *Boraginaceae* in 2018. The chasmothecium we sampled agrees with the descriptions of *G. asperifolii* published by Braun & al. (2018), and its sequence was highly consistent with those reported. According to the host plant records for this powdery mildew (Braun & al. 2018), *Bothriospermum chinense* represents a new host for *G. asperifolii*.

### Acknowledgments

This paper was supported by the National Natural Science Foundation of China (No. 31600513). We thank U. Braun (Institute of Biology, Martin Luther University, Halle, Germany) for valuable suggestions and critical comments on the manuscript and Prof. Zhimin Cao (Northwest A&F University, Yangling, China) and Prof. Susumu Takamatsu (Mycologist Emeritus, Tsu, Mie, Japan) for their expert reviews.

**Literature cited**

Braun U, Cook RTA. 2012. Taxonomic manual of the *Erysiphales* (powdery mildews). CBS Biodiversity Series 11. 707 p.

Braun U, Bradshaw M, Zhao TT, Cho SE, Shin HD. 2018. Taxonomy of the *Golovinomyces cynoglossi* complex (*Erysiphales, Ascomycota*) disentangled by phylogenetic analyses and reassessments of morphological traits. Mycobiology 46(3): 192–204. https://doi.org/10.1080/12298093.2018.1509512

Chen ZX. 1993. Fujian *Erysiphaceae*. Fuzhou, Fujian Science and Technology Press. (in Chinese).

Cook RTA, Inman AJ, Billings C. 1997. Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycological Research 101: 975–1002. https://doi.org/10.1017/S095375629700364X

Hirata T, Takamatsu S. 1996. Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37: 283–288. https://doi.org/10.1007/BF02461299

Liu TZ. 2010. Powdery mildew in Inner Mongolia. Hohhot, Inner Mongolia Science and Technology Press. (in Chinese).

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. Oxford University Press. New York.

Takamatsu S, Kano Y. 2001. PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. Mycoscience 42: 135–139. http://dx.doi.org/10.1007/BF02463987

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599. https://doi.org/10.1093/molbev/msm092

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513. https://doi.org/10.2144/000114018

Wang RS, Dou YX, Zhu KG. 2002. The notes on *Erysiphales* in the Guiqing Mountain of Gansu Province. Journal of Gansu Agricultural University 37(4): 416–420. (in Chinese). https://doi.org/10.3969/j.issn.1003-4315.2002.04.004

Wu MX, Wu MQ. 1991. Guizhou plant powdery mildew. Guizhou, Guizhou Science and Technology Publishing House. (in Chinese).

Zheng RY, Yu YN. 1987. Flora Fungorum Sinicorum. Volume 1 (*Erysiphales*). Beijing, Science Press. (in Chinese).